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Neuregulin 4 in Polycystic Ovarian Syndrome (PCOS) Phenotypes: A Key Role or Standby

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Abstract

Background: Neuregulin_4 (NRG4) is one of the adipokines members that synthesize adipose tissues. It has an activating effect on epidermal growth factor receptors (ErbB receptors). NRG4 has indirect effects on the hormonal environment through its interaction to ErbB receptors. Increased insulin resistance and chronic low-grade inflammation may be present when NRG4 levels are high in PCOS. Obesity and polycystic ovarian syndrome have recently gained a lot of attention. However, the literature on the connection between NRG4 and the PCOS phenotype is limited. Thus, this research aimed to identify neuregulin_4's function as a biomarker for insulin resistance in PCOS phenotypes.

Methods: A case-control study and included 140 female cases effect by different phenotypes of PCOS. Patients samples were collected at the reproductive fertility consultant of the Teaching Hospital for Obstetrics and Gynecology, Kerbala health directorate, Iraq. The outpatient clinic serum hormonal levels and insulin concentration were determined by the electrochemiluminescence immunoassay "ECLIA" system. Elisa system was used for the detection of Neuregulin-4 protein level.

Results: At the early age of participant NRG4 was increased significantly in all phenotypes of PCOS compared to control with a $P< 0.05$. interestingly, phenotype A was shown high level of NRG4 following phenotype C than phenotype D and phenotype B. Receiver Operator Characteristic Curves (ROC) analysis for NRG4 was performed and showed good diagnostic performers to word phenotype A.

Conclusion: Females with phenotype A have a higher level of NRG4 than other phenotypes, which could be attributable to the more pronounced metabolic abnormalities in this phenotype.

Keywords: Metabolic disturbances, Neuregulin 4, Polycystic Ovarian Syndrome Phenotypes.

Introduction

As a result of the frequency and prevalence of metabolic disorder like insulin resistance (IR), (1) and obesity (2), are rising worldwide, posing significant public health concerns and burdens. Consequently, to proteins that could regulate metabolic homeostasis, there is a rising interest (3). The majority of studies have focused on adipose tissue-derived factors, known as adipokines (4). Adipose tissue has been shown to act as an endocrine organ and to release a wide variety of adipokines. Excessive production of adipokines has been linked to insulin resistance and related disorders like obesity, type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and polycystic ovarian syndrome (PCOS) (5).

Hyperinsulinemia, elevated androgen levels, and obesity are hallmarks of polycystic ovary syndrome (PCOS), a prevalent endocrine condition (6). Those who suffer from this condition are more likely to develop cardiovascular disease and metabolic syndrome (7). in 2012 the National Health and Medical Research Council (NHMRC), Evidence-based Methodology Workshop Panel on Polycystic Ovary Syndrome considered Rotterdam criteria most commonly diagnosed (8). The widely used Rotterdam criteria were mostly upheld by the NHMRC PCOS-2018 recommendations (9). This criterion required two out of the three following features to occur (after the exclusion of related disorders): ovulatory dysfunction

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(oligo/ amenorrhea) (OD), clinical and/or biochemical hyperandrogenism (AH), or polycystic ovaries morphology (PCOM), It has four phenotypes A (AH, OD, and PCOM), phenotype B (AH and OD), phenotype C (AH and PCOM) and phenotype D (OD and PCOM) (10). The distribution of PCOS phenotypes is variable and highly dependent on the method used to identify the population (11).

Neuregulin 4 (NRG4) is a protein predominantly present in brown adipose tissue cells and encoded by the NRG4 gene (12).NRG4 is one of the adipokines members that synthesize adipose tissues, it acts to active the epidermal growth factor receptor (EGFR known as ErbB receptors) extracellular ligands and binds specifically to ErbB4 activation that begins cytosolic tyrosine phosphorylation to formation signaling between cells (13), in particular the endocrine and paracrine signaling pathways, NRG4 serves a variety of other roles in the human body ,in addition to its role in optimizing cell energy metabolism and reducing inflammation and apoptosis (14). The target of this research is to identify neuregulin_4's function as a biomarker for insulin resistance in PCOS phenotypes.

Materials and Methods

Study design

The current case-control study included 210 women's:140 females with PCOS divided into four phenotypes (A, B, C and D), and 70 control women. Patients with PCOS were gathered from the outpatient clinic and reproductive fertility consultant at the Teaching Hospital of Obstetrics and Gynecology in the Kerbala health directorate in Iraq. Normoandrogenic, hirsutism-free, regular, ovulatory menstrual cycle history, and ultrasound evidence of morphologically normal ovaries characterized the women in the control group. The Rotterdam criteria from 2003 were used to get the PCOS diagnosis. Clinical and/or biochemical hyperandrogenism, oligo/anovulation, and ultrasound evidence of polycystic ovaries (>12 follicles 2-9 mm in diameter or ovarian volume >10 mL in at least one ovary) were considered diagnostic of polycystic ovary syndrome (15). Criteria for exclusion Women with a history of taking any other medication (lipid-lowering agents, contraceptive pills, ovulation stimulation, corticosteroids, antidiabetic, and antihypertensive medications), as were women with autoimmune disease, T2DM, thyroid disease, hyperprolactin, cardiovascular disease, hypertension, chronic liver failure, chronic renal failure, and malignant diseases. All steps were taken in compliance with the rules and regulations that were in effect at the time. Each woman in the study was given a thorough interview that included questions about her background, her family's background, her demographics, and a clinical assessment in the lab.

Study Protocol

A thorough physical was performed on each and every woman. If a patient scores four or more points based on the race-specific modified Ferriman-Gallwey scale, they are considered to have clinical hyperandrogenism-hirsutism (11). The presence of acne and male-pattern baldness was assessed. Oligo/amenorrhea was defined as having fewer than six menstrual cycles per year. The formula for calculating body mass index (BMI) is body weight in kilograms divided by height in meters squared (kg/m2). For women with abdominal obesity, waist circumference was measured in the standing position, and WHR was calculated as the ratio of waist (cm)to hips (cm). Ovarian volume greater than 10 ml on ultrasonography and fewer than 12 follicles measuring 2 to 9 mm are indicative of polycystic ovarian morphology (PCOM). Morning samples of fasting blood were collected between the second and fourth day of the cycle.

Biochemical Analyses

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid-stimulating hormones (TSH), and insulin concentration were determined by the electrochemiluminescence immunoassay

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"ECLIA" system (Cobas e 411, Roche Diagnostic, Germany). Fasting blood glucose was determined by a Clinical chemistry analyzer(Monarch 240, Biorex Diagnostic, United Kingdom).serum lipid concentration ((total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL)and triglycerides (TG)) fully automatic chemistry analyzer(SMART-120, Geno TEK, United States of America).serum free testosterone hormone fully -auto chemiluminescence immunoassay analyzer((MAGLUMI 600, Snibe Diagnostic, Germany) The measurement of neuregulin 4 concentration (ng/mL), competition principle by Sandwich enzyme immunoassay.

Calculations

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [fasting sugar (mg/dl) x fasting insulin (µU/ml) /405] (16).

Statistical Analysis

For this project's data analysis, we relied on IBM's SPSS Statistics 28.0 (Chicago, IL, USA) and the Real Statistics Resource Pack for Excel 2016 (Release 7.2) for Mac. From the years 2013 until 2020, copyright. Using the Shapiro-Wilk test as a numerical measure of normality, the distribution of the data was examined. Non-conditional logistic regression was used to estimate the association between the analyzed factors and the presence of PCOS using odds ratios (ORs) and a 95% Confidence Interval Range. A *P*-value less than or equal to 0.05 was considered statistically significant. Using receiver operating characteristic (ROC) analysis, for really important cases, the ideal threshold was shown to have both high specificity and sensitivity.

Results

A total of 210 participants were included in this study, seventy samples were collected from normal participants as a healthy control, the total number of PCOS is 140. In the PCOS group 69 (49.28%) females had phenotype A. 20 (14.2%) females had phenotype B.23(16.42%) females in phenotype C, and 28 (20%) females had phenotype D. The clinical features of the investigated group were analyzed. The present and absent of acne were about (57.85%, and 42.14%) respectively. Also, alopecia was present and absent (83.57% and 16.42%) respectively. The analysis of ovulatory dysfunction showed that 117 have oligo-amenorrhea that represents groups (A, B, and D) of patients while 23 have regular mens group (C).The investigation of Ovarian Morphology showed that 120 patients have PCOS groups (A, C, and D) and 20 have a normal group (B) of patients .The demographic, laboratory, and metabolic parameters of the study group's population were shown in Table (1).

Illustrated the mean level of NRG4 in the Patients and control groups according to Age. In group 1 the age range of (18- 27) years, NRG4 was increased significantly in patients compared to control with a *P*-value < 0.05 but in group 2 the range of age groups (equal and more than 28) years was non-significant in patients (Fig. 1).

In comparison to healthy controls, patients with polycystic ovary syndrome had a higher NRG4 range level., where phenotype A is more increasing following phenotype C than phenotype D and phenotype B (Fig. 2).

It was found that NRG4 showed highly significant differences in the PCOS phenotype group and represented a risk factor, Table 2. NRG4 was represented as a risk factor in Phenotype A followed by phenotype (C, D and B).

The AUC had extremely significant *P*-values (below 0.05). Results of the Sensitivity & Specificity were confirmed using Youden's J statistics to the parameters. Table 3 displays the results of an investigation of the ROC curves for the NRG4 PCOS phenotypic groups vs the control group.

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Table 1. The demographic, laboratory, metabolic parameters, insulin resistance index, and biomarkers between PCOS patients and controls.

	PCOS		Control		Normal value
	(Mean ± SD)	Median (Min-Max)	(Mean ± SD)	Median (Min-Max)	
Demographic Parameters					
Age (years)	(24.66±4.89)	24(18-44)	(27.60±4.10)	28(21-43)	
WHR	(0.84 ±0.05)	0.84(0.70-0.95)	(0.79±0.05)	0.78(0.72-0.94)	
BMI	(28.98±5.4)	28.6(19.4-41.6)	(23.64±1.96)	23.6(19.2-29)	
Hormonal Parameters					
LH	(9.60±4.04)	8.94(2.12-21.70)	(4.40±1.15)	4.40(2.14- 6.94)	1.5-8 m.lu/mL
FSH	(5.76±1.53)	5.50(2.90- 10.32)	(8.64±1.53)	8.74 (5.73-11.58)	2.9-12 m.lu/mL
LH/FSH	(1.79±0.79)	1.68(0.45- 4.06)	(0.51±0.12)	0.49(0.31- 0.76)	/
Prolactin	(17.46±6.66)	16.83(5.31-30)	(15.81±5.53)	15.80(4.96-25)	5-35 ng/mL
TSH	(2.24±0.78)	2.26(0.63-4.05)	(2.30±0.48)	2.26(0.80- 3.20)	0.27-4.2 ulU/mL
FT	(1.96±1.05)	1.70(0.55-4.90)	(0.83±0.44)	0.91(0.12-1.52)	<4.2 pg/ mL
Lipid Profile					
Cholesterol	(158.49±29.63)	161(91-237)	(156.41±22.44)	161(110-198)	0-200 mg/dl
Triglycerides	(86.41±28.55)	84(35-170.58)	(74.43± 15.13)	75.93(40-112)	0-200 mg/dl
LDL	(88.66±26.73)	86.7(39.4-158.78)	(70.59±16.31)	71(40-99.60)	0-160 mg/dl
HDL	(54.98±7.11)	55(40.31-74)	(65.83±4.86)	64.56(58-77)	40-80 mg/dl
Insulin Resistance Index					
HOMA-IR	(3.17±0.94)	3.10(1.09-5)	(1.38±0.33)	1.29(1.02-2.40)	/
Proposed Biomarkers for PCOS Phenotypes					
NRG4	(8.87±3.03)	8.90(2.33-17.39)	(7.09±4.11)	5.40(2.40-9.643)	/

Data expressed as mean ± standard deviation (SD) and median (interquartile range); BMI: body mass index; WHR: waist hips ratio; FT: free testosterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; TSH: Thyroid-stimulating hormone; NRG4: Neuregulin4.

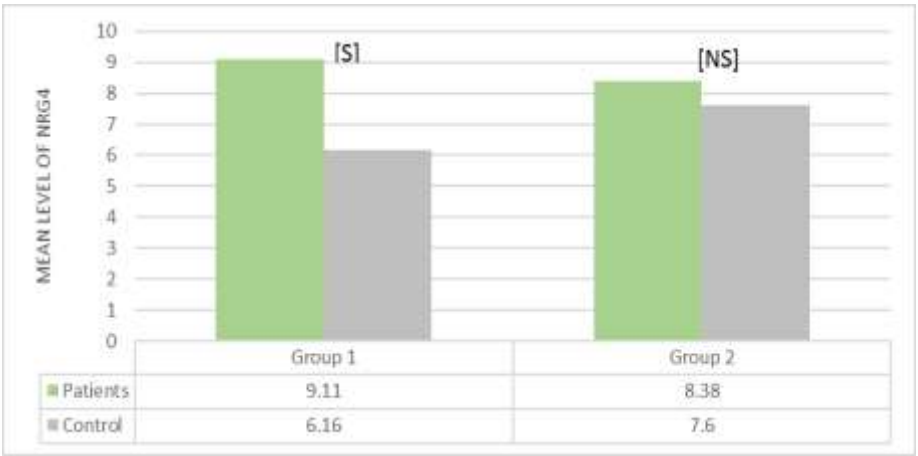

Fig. 1. Difference Between Mean Levels of NRG4: Neuregulin 4 with Age Group (1) and Group (2) years $P<0.05$ considered significantly different; [S]= Significant, [NS]= non-significant.

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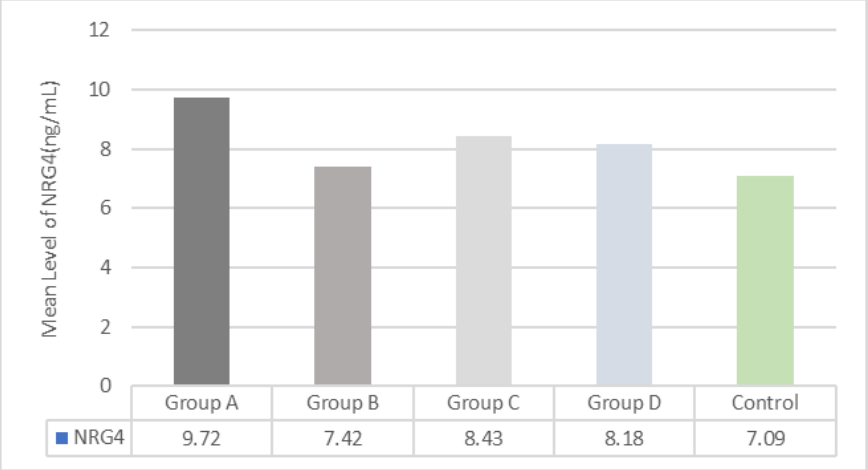

Fig. 2. Mean differences of serum level NRG4: Neuregulin 4 for PCOS Phenotype compared to the control group.

Table 2. The Multinomial Logistic Regression of PCOS Phenotype Groups with NRG4.

Variable	Groups	OR (Lower – upper)	P value
NRG4	**Group A**	1.262(1.132-1.407)	<0.001[S]
	Group B	1.033(0.883-1.208)	0.023[S]
	Group C	1.133(0.981-1.309)	0.001[S]
	Group D	1.109(0.969-1.270)	0.004[S]
	Control Group	1[a]	-

P<0.05 considered significantly different; [S]= Significant, [NS]= Nonsignificant ;1[a]: reference category is Control; NRG4: Neuregulin 4.

Table 3. Receiver operating characteristic curve showing sensitivity and specificity of NRG4 in PCOS Phenotypes.

Variable	AUC	P value	Sensitivity %	Specificity %	Cut off	Youden index	CI %
Group A	0.86	<0.001	90%	89.7%	7.68	0.79	0.77-0.95
Group B	0.66	0.038	70%	70%	5.91	0.4	0.50-0.82
Group C	0.74	0.001	60.9%	85%	6.95	0.54	0.61-0.87
Group D	0.77	<0.001	82.1%	70%	5.85	0.52	0.65-0.89

Discussion

This is the first report comparing NRG4 serum concentrations among PCOS phenotypes, as far as we are knowledgeable. We observed a higher NRG4 in phenotype A compared to the other phenotypes, and our result is of high risk and significance. Phenotype A, a classic phenotype linked to an increased risk of obesity, type 2 diabetes, insulin resistance, coronary heart disease, and other metabolic disorders, was confirmed to be linked to an increased risk of these diseases in previous research (10) .NRG4 is one of the members of the adipokines family that is synthesized and secreted by adipose tissues; it acts to activate the epidermal growth factor receptor (EGFR). Because EGFR deficiency can result in ineffective LHRH secretion, the EGFR plays a crucial role in the healthy function of luteinizing hormone-releasing hormone (LHRH), which is responsible for normal female pubertal development (17). The regulation of energy metabolism is mostly

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dependent on adipose tissue, an endocrine organ that is an essential role. Energy is stored in white adipose tissue as triacylglycerols, while brown adipose tissue uses fatty acids as fuel to keep the body at a constant temperature. NRG4 is an endocrine factor that is abundant in brown fat and is part of a family of proteins called epidermal growth factors (18). We also observed an increase in serum concentrations of NRG4 in phenotypes C, D, and B. Theoretically, Patients with polycystic ovary syndrome (PCOS) may have an increased neuregulin level due to NRG4-EGFR binding difficulties (19). However, additional research is required to corroborate this hypothesis. Serum levels of NRG4 were found to be elevated in PCOS by Temur et al. (2017), high levels of NGR4 were positively correlated with fasting blood glucose, insulin, HOMA-IR, and high-sensitivity C-reactive protein (hs-CRP) in the study population. Independent markers of NGR4 level were found to be HOMA-IR and hs-CRP. (19).

Using multiple regression analysis, Kurek Eken et al. (2019) demonstrated that serum NRG4 level was independently associated with BMI (18). Obesity was the most influential factor in NRG4 secretion in PCOS patients, and weight management was the key to resolving metabolic abnormalities and fertility problems related to PCOS (20) Meta-analysis highlights the importance of weight intervention and lifestyle modification for the treatment of adolescent PCOS because of the shared metabolic disorders of obesity and PCOS, such as hyperandrogenism and hyperinsulinemia with insulin resistance (21-23). The study by Cao et al in 2021 had similar results to the Kurek Eken et al study. Furthermore, the study showed that NRG4 levels could be brought up to par with the healthy controls by adopting a lifestyle intervention comprising dietary modification, increased physical activity, and reduced sedentary behaviour for as little as a year. (20).

Our investigation of NRG4 levels in the PCOS phenotype revealed a statistically significant difference between the four phenotype groups studied. The females with phenotype A have a higher concentration of NRG4 than the control group. Consequently, metabolic disturbances may be more severe in this phenotype than in other phenotypes. Further research on PCOS is required to reduce adiposity, as are future gene polymorphism studies examining NRG4 levels.

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Conflicts of interest
There are no conflicts of interest.

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References
1. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018;20(2):12.
2. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metabolism. 2019;92:6-10.
3. Czerwińska M, Czarzasta K, Cudnoch-Jędrzejewska A. New Peptides as Potential Players in the Crosstalk Between the Brain and Obesity, Metabolic and Cardiovascular Diseases. Front Physiol. 2021;12:692642.
4. Zorena K, Jachimowicz-Duda O, Ślęzak D, Robakowska M, Mrugacz M. Adipokines and Obesity. Potential Link to Metabolic Disorders and Chronic Complications. Int J Mol Sci. 2020;21(10):3570.
5. Landecho MF, Tuero C, Valentí V, Bilbao I, de la Higuera M, Frühbeck G. Relevance of Leptin and Other Adipokines in Obesity-Associated Cardiovascular Risk. Nutrients. 2019;11(11):2664.
6. Al-Lami HB, Al-Tu'ma F J, Al-Safi WG. Association between anti-Müllerian hormone and other biomarkers with ovarian function in polycystic ovarian syndrome of Iraqi women. J Contemp Med Sci. 2020;6(4).

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7. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics. Metabolism. 2019;92:108-120.
8. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-1618.
9. Moran LJ, Tassone EC, Boyle J, Brennan L, Harrison CL, Hirschberg AL, et al. Evidence summaries and recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome: Lifestyle management. Obes Rev. 2020;21(10):e13046.
10. Polak AM, Adamska A, Krentowska A, Łebkowska A, Hryniewicka J, Adamski M, Kowalska I. Body Composition, Serum Concentrations of Androgens and Insulin Resistance in Different Polycystic Ovary Syndrome Phenotypes. J Clin Med. 2020;9(3):732.
11. Mumusoglu S, Yildiz BO. Polycystic ovary syndrome phenotypes and prevalence: Differential impact of diagnostic criteria and clinical versus unselected population. Curr Opin Endocr Metab Res. 2020;12:66–71.
12. Tutunchi H, Ostadrahimi A, Hosseinzadeh-Attar MJ, Miryan M, Mobasseri M, Ebrahimi-Mameghani M. A systematic review of the association of neuregulin 4, a brown fat-enriched secreted factor, with obesity and related metabolic disturbances. Obes Rev. 2020;21(2):e12952.
13. Gumà A, Martínez-Redondo V, López-Soldado I, Cantó C, Zorzano A. Emerging role of neuregulin as a modulator of muscle metabolism. Am J Physiol Endocrinol Metab. 2010;298(4):E742-50.
14. Liu Y, Chen M. Neuregulin 4 as a novel adipokine in energy metabolism. Front Physiol. 2023;13:1106380.
15. Chen W, Pang Y. Metabolic Syndrome and PCOS: Pathogenesis and the Role of Metabolites. Metabolites. 2021;11(12):869.
16. Bahadur A, Verma N, Mundhra R, Chawla L, Ajmani M, Sri MS, Arora S. Correlation of Homeostatic Model Assessment-Insulin Resistance, Anti-Mullerian Hormone, and BMI in the Characterization of Polycystic Ovary Syndrome. Cureus. 2021;13(6):e16047.
17. Ayoob LA. Evaluation of serum Neuregulin4 level in Polycystic ovary syndrome patients. Eurasian Med Res Period. 2022;13:7-10.
18. Kurek Eken M, Sahin Ersoy G, Yayla Abide C, Sanverdi İ, Devranoglu B, Kutlu T, Çevik Ö. Association between circulating neuregulin 4 levels and metabolic, aterogenic, and AMH profile of polycystic ovary syndrome. J Obstet Gynaecol. 2019;39(7):975-980
19. Temur M, Calan M, Akşit M, Yılmaz Ö, Çift T, Akselim B, et al. Increased serum neuregulin 4 levels in women with polycystic ovary syndrome: A case-control study. Ginekol Pol. 2017;88(10):517-522.
20. Cao S, Hu Y. Effects of serum irisin, neuregulin 4, and weight management on obese adolescent girls with polycystic ovary syndrome. Biosci Rep. 2021;41(9):BSR20211658.
21. Li L, Feng Q, Ye M, He Y, Yao A, Shi K. Metabolic effect of obesity on polycystic ovary syndrome in adolescents: a meta-analysis. J Obstet Gynaecol. 2017;37(8):1036-1047.
22. Ariaee N, Farid R, Shabestari F, Shabestari M, Jabbari Azad F. Trace Elements Status in Sera of Patients with Allergic Asthma. Rep Biochem Mol Biol. 2016;5(1):20-25.
23. Shenta A, Saud K, Al-Shawi A. Assessment the Correlations of Hormones, Lipid Profiles, Oxidative Stress, and Zinc Concentration in Iraqi Women with Polycystic Ovary Syndrome. Rep Biochem Mol Biol. 2020;9(3):270-277.

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